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Effects of mass inoculation on induced oleoresin response in intensively managed loblolly pine

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Summary Oleoresin flow is an important factor in the resistance of pines to attack by southern pine beetle, *Dendroctonus frontalis* Zimm., and its associated fungi. Abiotic factors, such as nutrient supply and water relations, have the potential to modify this plant–insect–fungus interaction; however, little is known of the effects of inoculation with beetle-associated fungi on oleoresin flow. We observed that constitutive and induced resin yield in loblolly pine, *Pinus taeda* L., were affected by either fungal inoculation (with the southern pine beetle-associated fungus *Ophiostoma minus* (Hedgcock) H. & P. Sydow) or silvicultural treatment. The effects of mass wounding (400 wounds m^{-2}) and mass wounding and inoculation with *O. minus* were assessed by comparison with untreated (control) trees. The treatments were applied to trees in a 2×2 factorial combination of fertilizer and irrigation treatments. Fertilization did not significantly affect constitutive resin yield. Even as long as 105 days post-treatment, however, mass-inoculated trees produced higher induced resin yields than control or wounded-only trees, indicating a localized induced response to fungal inoculation. We noted no systemic induction of host defenses against fungal colonization. Although beetles attacking previously attacked trees face a greater resinous response from their host than beetles attacking trees that had not been previously attacked, the effect of an earlier attack may not last more than one flight season. Despite mass inoculations, *O. minus* did not kill the host trees, suggesting that this fungus is not a virulent plant pathogen.

Keywords: cofactor, Dendroctonus frontalis, fertilization, irrigation, Ophiostoma minus, pathogenicity, Pinus taeda, resistance, southern pine beetle.

Introduction

Loblolly pine, *Pinus taeda* L., is the most widely planted forest tree in the southeastern United States, established on about 19 million ha (Baker and Langdon 1990, Schultz 1997). This tree is increasingly being managed intensively for rapid growth through site preparation, weed control, fertilization and stand thinning (Wear and Greis 2002).

Southern pine beetle (SPB), *Dendroctonus frontalis* Zimm., is the most important pest of loblolly pine in the United States and causes more damage than any other forest insect in the southern USA (Drooz 1985). During eruptive periods, when mass attack by beetles overwhelm host defenses, SPB can kill large numbers of healthy pines and have substantial economic and ecological impacts (Coulson 1980). In addition, SPB-attacked trees are challenged with infection by *Ophiostoma minus* (Hedgcock) H. & P. Sydow, a fungus commonly transmitted by SPB, which infects functional sapwood tissue, causing blue-stain. This fungus has an important role in SPB ecology (Klepzig et al. 2001). Other fungal associates of SPB, *Ophiostoma ranaculosum* (J.R. Bridges & T.J. Perry) Hausner (syn. *Ceratocystiopsis ranaculosus*; Jacobs and Kirisits 2003) and *Entomocorticium sp. A*, have negligible impacts on beetle-infested trees (Klepzig et al. 2001, Lombardero et al. 2003).

Initial tree defense against attack by this insect is through the exudation of oleoresin into the holes the adult beetles bore through the bark (Hodges et al. 1979). Beetle attack can be successfully resisted when sufficient oleoresin is produced at each wound to either entomb or "pitch-out" the insect (Trapp and Croteau 2001). It is generally accepted that no tree of suitable size and species is immune to SPB attack at high enough densities (Strom et al. 2002); however, resistance of individual trees varies and environmental attributes that affect oleoresin production can have substantial impacts on the success of beetle attack. Forest management activities that impact oleoresin production may therefore have effects on pest management. Understanding the roles of SPB and its fungal associates in relation to tree response to attack is important.

Means of investigating the effects of SPB invasion without simultaneous fungal infection have not been devised. Moreover, controlled infestation of healthy trees with SPB under field conditions is difficult to achieve (Cook and Hain 1987).

Thus, experiments to assess effects on host trees of SPB attack are often limited to artificial wounding and fungal inoculation. Previous work on other systems has demonstrated the feasibility and utility of mass inoculation to test host responses to beetles and vectored fungi (Horntvedt et al. 1983, Raffa and Berryman 1983, Christiansen et al. 1985, Solheim et al. 1993, Guérard et al. 2000, Krokene and Solheim 2001, Langstrom et al. 2001, Lieutier 2002). For this study, we tested whether fungal inoculation has lasting effects on resin defenses in loblolly, as has been reported previously for both loblolly and shortleaf pine *Pinus echinata* P. Mill. (Cook and Hain 1987) and for Norway spruce *Picea abies* (L.) Karst. (Christiansen et al. 1999). We also sought to determine whether such effects change with the physiological status of the host tree (in this case, as affected by silvicultural treatment). We designed our study to test the simultaneous effects of silviculturally manipulated abiotic factors (fertilization, irrigation, thinning) and artificial mass wounding and inoculation with *O. minus* on constitutive and induced oleoresin defenses in loblolly pine. We measured these effects by comparing oleoresin production in wounded or wounded and inoculated trees compared with untreated (control) trees. We also quantified a secondary defense of pines against *O. minus*, by measuring the extent to which the inoculated fungus colonized tree tissue. Finally, we tested the capacity of loblolly pine for induced systemic resistance to bark beetle-associated fungi following mass inoculation, a phenomenon previously observed in Norway spruce (Krokene et al. 1999), Scots pine *Pinus sylvestris* L. (Krokene et al. 2000), Monterey pine *Pinus radiata* D. Don. (Bonello et al. 2001) and Austrian pine *Pinus nigra* Arnold (Bonello and Blodgett 2003).

Materials and methods

We investigated the defense responses of loblolly pine, grown under different management intensities, against mass wounding and inoculation with *O. minus* (as a surrogate for mass attack by SPB), using techniques modified from work in other systems (Krokene and Solheim 2001). Our investigations were conducted at the Southeast Tree Research and Education Site (SETRES) in Scotland County, NC, and in the pine forests of Camp Beauregard, U.S. Army National Guard land located in Rapides Parish, LA.

North Carolina

The SETRES site (34°48′ N, 79°12′ W) was established in 1992 in an 8-year-old plantation to study the effects of optimal irrigation and fertilization on the growth of loblolly pine. The experimental design was a 2 × 2 randomized complete block with two treatments: irrigation (irrigated and unirrigated) and fertilization (fertilized (N,P,K + micronutrients) and unfertilized). Each plot consisted of a 0.25-ha block of trees separated from other plots by a fire line with a belowground root barrier. A detailed description of the study site and treatments can be found in Albaugh et al. (1998). At the time of our study, the trees were 16 years old.

We selected a total of eight codominant trees in each of the 16 plots (4 plots per silvicultural treatment, for a total of eight trees in each inoculation or wounding treatment per silvicultural treatment). On June 13, 2000, we subjected two trees in each plot to one of the following treatments: wounding (400 wounds m^{-2}); and woulding + inoculation with *O. minus*. Two untreated trees in each plot were selected as controls. To establish a relatively uniform experimental surface, the loose outer bark of the trees was scraped off before wounding. We inflicted all wounds in a 1-m-deep band on each tree, beginning 0.8 m above ground. An approximately 15-cm-wide band of bark in the middle of the wounded band was left unwounded and uninoculated to facilitate subsequent resin sampling. For each wound, the tree was struck with a 4-mm-diameter increment hammer and a bark–phloem plug extracted, exposing the cambium–xylem interface. For inoculated trees, we inserted into each wound a 4-mm-diameter × 3-mm-thick disk of malt extract agar colonized by *O. minus* hyphae (from a 2–4-week-old culture of *O. minus* isolated from a female SPB collected in the Bankhead National Forest in Alabama, USA). We sealed all wounds, with or without fungal inoculation, with a small piece of 4-mm diameter wooden dowel to limit infection by feral spores and to simulate natural wound closing at beetle attack sites.

We sampled all study trees for 24-h oleoresin yield at 1-day pre-wounding/inoculation and at 1, 15 and 105 days post-wounding/inoculation. We measured resin yield by removing an 8-mm-diameter bark–phloem plug and attaching a small metal tray to the tree to direct the exuded resin into a vial (Lorio and Sommers 1986). We sampled each tree once on each sampling date. After 24 h, we weighed all the vials and calculated the resin yield (g resin day^{-1}).

Parallel with the above treatments, we inoculated each study tree twice with single point inoculations of *O. minus* (but otherwise as described above) at approximately 1 m above the mass wounding/inoculation site, on opposite sides of the tree, 21 days after the mass wounding/inoculation. We sampled the point inoculations on post-treatment day 106.

On post-treatment day 106, when all the resin samples had been collected, we scraped the remaining outer bark (rhytidome) and inner bark phloem from an area of approximately 15 × 35 cm of wounded/inoculated bark on each tree, and traced the area of resinous lesions displayed on the sapwood surface inside an area 10.6 × 27.5 cm onto acetate overhead transparency sheets. We measured the area of all resinous lesions traced on each sheet with a digital planimeter as an estimate of the extent to which fungus had colonized tree tissue.

Louisiana

In 2001, we studied two loblolly pine sites in Camp Beauregard (31°23′ N, 92°24′ W), LA. Both sites were established in 1975. One site was thinned in 1990, and a plot containing 30 codominant trees was established. The second site was unthinned and contained a plot of 60 trees (30 codominant, 30 intermediates). Within each site, previous investigators had chosen dominant or codominant trees, half of which were fertilized and the other half not. Fertilization was carried out in

spring of 1997 and 1998 with diammonium phosphate. Details of the fertilization treatments can be found in Lombardero et al. (2000).

We selected 27 codominant trees at each site that appeared to be healthy and free of insect or disease damage. In June, 2001, we subjected each tree to one of the following three treatments: wounding (400 wounds m^{-2}), wounding + inoculation with *O. minus*, or untreated (control). We used the same techniques and same fungal isolate as described for the NC site.

We sampled all trees for oleoresin yield: 1 day before inoculation, as well as 1, 15, 30 and 105 days post inoculation. One year after the initial wounding-inoculation treatment, we conducted an additional resin sampling. Resin sampling methods followed those described above for the NC site.

Statistical analyses

We evaluated data from both experiments by a repeated-measures analysis of variance (ANOVA), in which sampling time was treated as a repeated factor with four levels. Sphericity tests (Anderson 1958) were applied to determine if the covariance matrix among orthogonally transformed components of resin yield, measured at the four times, satisfied the Huynh-Feldt condition (Huynh and Feldt 1970). The SETRES data passed the sphericity test ($P = 0.1623$), but the Camp Beauregard data did not ($P < 0.0001$). For consistency, we used multivariate tests for within-tree (between-sampling time) effects and interactions that involved these effects. We applied four tests to the data: Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Maximum Root (Morrison 1976). We tested between-tree effects and their interactions, with conventional type I ratios computed from type III sums-of-squares. We computed least-squares means and their standard errors (corrected for other terms in the model) for significant effects and interactions. We made multiple-comparisons, with probabilities adjusted by the Tukey-Kramer approach (Kramer 1956) and an experiment-wide alpha level of 0.05, to determine which pairs of means were significantly different. We performed all computations with proc GLM in SAS 8.2 (1999, SAS Institute, Cary, NC).

The lesion size data from the SETRES experiment were highly skewed, with a few large outlying values. A fourth-root transformation was applied before ANOVA to correct the skewness and reduce the weight of these outlying observations. The transformed data were analyzed by 3-way factorial analysis of variance, with irrigation, fertilization and simulated insect attack (wounding or wounding plus inoculation) as main effects. Main effects and their interactions were tested with *F* ratios computed using type III sums-of-squares. Least-squares means and their standard errors, corrected for other terms in the model, were computed for significant effects and interactions. Multiple-comparisons were conducted as described above. Because lesion size at Camp Beauregard differed by inoculation/wounding treatment (fungal inoculations produced the largest lesions) but not by silvicultural treatment, lesion size at this site was not analyzed.

Results

At neither experimental site did any treated trees die. At both sites, the wounding and inoculation treatments resulted in resinous lesions and affected resin yield.

North Carolina

Resin yield Resin yields of controls and treatment groups were equal before the wounding and inoculation treatments. One day after treatment, resin yields of the wounded and wounded + inoculated trees were significantly reduced—about one fifth that of the controls (Figure 1). After 15 days, resin yields of trees, that were wounded and inoculated, were significantly higher than, and about twice that of, the wounded only trees. At the conclusion of the experiment, 105 days after treatment, resin yield in the wounded and inoculated trees remained significantly higher (about 3×) than that of the controls. Resin yield for the wounded only group did not differ significantly from the controls. Trees subjected to two inoculations only behaved like the controls throughout the experiment. Neither fertilization nor irrigation, nor any of their interactions, had a significant effect on resin yield; however, the sampling time × treatment interaction was clearly significant ($F_{9, 112} = 5.86$, $P < 0.0001$) (Table 1). For within-tree effects and interactions, all four multivariate tests yielded consistent results, so only the Wilks' Lambda results are displayed (Table 1).

Lesion size In all but one of the silvicultural treatments at the SETRES site, lesions formed in trees that were mass wounded and inoculated with *O. minus* were significantly larger than those formed in trees that were mass wounded without inoculation (Figure 2). Lesions formed as a result of fungal inoculations in trees that were both fertilized and irrigated were

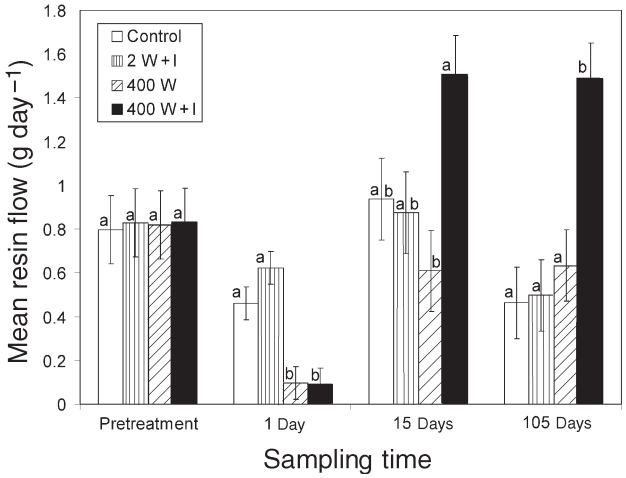

Figure 1. Effects of wounding (W) and inoculation with *Ophiostoma minus* (I) on the yield of oleoresin from a 1.25-cm wound in loblolly pine at the SETRES site, North Carolina. Trees were either wounded and inoculated twice (2 W + I), wounded 400 times (mass wounded, 400 W), or mass wounded and inoculated (400 W + I). Error bars are standard errors. Identical letters indicate means that are not statistically different within each sampling time.

Table 1. Repeated-measures ANOVA of resin flow data from trees at the SETRES site, North Carolina. (a) Multivariate (MANOVA) tests for within-tree (between-sampling time) effects and interactions. (b) Tests for between-tree effects and interactions.

(a) Within-tree effects

Source	Wilks' Lambda	*F*	$df_{1,2}$	*P*
Time	0.3972	23.28	3, 46	< 0.0001
Time × block	0.8260	1.02	9, 112.1	0.4301
Time × irrigation	0.9605	0.63	3, 46	0.5986
Time × fertilization	0.9930	0.11	3, 46	0.9553
Time × treatment	0.3913	5.86	9, 112.1	< 0.0001
Time × irrigation × fertilization	0.9880	0.19	3, 46	0.9047
Time × irrigation × treatment	0.8648	0.77	9, 112.1	0.6480
Time × fertilization × treatment	0.9422	0.31	9, 112.1	0.9708

(b) Between-tree effects

Source	df	Type III SS	MS	*F*	*P*
Block	3	2.5151	0.8384	1.34	0.2713
Irrigation	1	1.2198	1.2198	1.95	0.1685
Fertilization	1	0.2878	0.2878	0.46	0.5003
Treatment	3	6.5221	2.1740	3.48	0.0228
Irrigation × fertilization	1	0.4113	0.4113	0.66	0.4209
Irrigation × treatment	3	0.8528	0.2843	0.46	0.7146
Fertilization × treatment	3	0.3028	0.1009	0.16	0.9216
Error	48	29.9508	0.6240		

significantly longer than lesions formed in trees that were fertilized but not irrigated (Figure 2). Lesions resulting from point inoculations outside the mass wounding area did not significantly differ according to mass wounding or inoculation treatment (data not shown).

Louisiana

Resin yield Control and treated trees had similar resin yields before treatment. One day after treatment, both wounded and wounded + inoculated trees had significantly lower resin yields than control trees (Table 2, Figure 3). Both 15 and 105 days after treatment, resin yields in the wounded only trees did not significantly differ from the controls, whereas the wounded + inoculated trees exhibited significantly higher resin yields than trees in the other treatments (Figure 3). One year post-treatment, resin yields did not significantly differ between control, mass wounded and mass wounded + inoculated trees at Camp Beauregard. Neither silvicultural nor inoculation treatments significantly affected resin yield 1 year after treatment (Table 1). Neither thinning nor fertilization significantly affected resin yield (but the time × thinning × treatment interaction was almost significant, $P = 0.05$). As at the SETRES site, we found a significant sampling time × treatment interaction. Mean resin yields were generally three to four times higher than at SETRES (Figure 2).

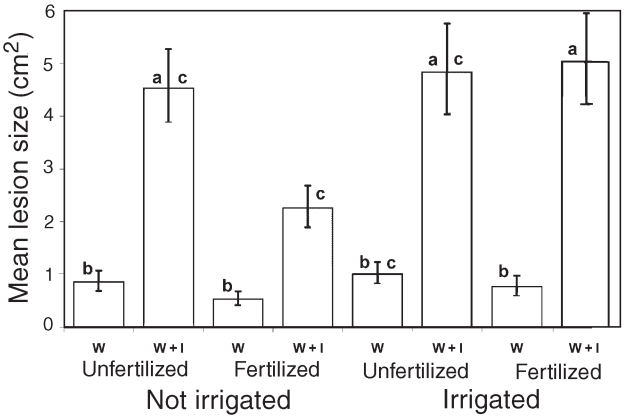

Figure 2. Effects of irrigation, fertilization and treatment (W = wounded only; W + I = wounded + inoculated with *Ophiostoma minus*) on mean lesion size in loblolly pine at the SETRES site, North Carolina. Error bars are standard errors. Both means and standard errors are back-transformed from the least-squares estimates derived from the ANOVA of fourth-root-transformed data. Identical letters indicate means that are not statistically different.

Discussion

Mass-inoculated trees produced higher resin yields than control or wounded only trees within 15 days of treatment and as late as 105 days after treatment. Resin yield thus responded positively and strongly to fungal inoculation. This phenomenon was consistent in two forests and years, and in trees with different constitutive resin yields. We do not know whether resin flow was affected beyond the inoculated area of the bole. The ability of fungal inoculation to stimulate resin flow was noted previously (Hepting 1947, Popp et al. 1991), but never in the context of a mass inoculation and simulated beetle attack.

Table 2. Repeated-measures ANOVA of resin flow data from the Camp Beauregard site, Louisiana. (a) Multivariate (MANOVA) tests for within-tree effects and interactions. (b) Tests for between-tree effects and interactions.

(a) Within-tree effects

Source	Wilks' Lambda	*F*	df_1	*P*
Time	0.2710	38.56	3, 43	< 0.0001
Time × thinning	0.9675	0.48	3, 43	0.6973
Time × fertilization	0.9666	0.50	3, 43	0.6872
Time × treatment	0.2314	15.46	6, 86	< 0.0001
Time × thinning × fertilization	0.9395	0.92	3, 43	0.4377
Time × thinning × treatment	0.7528	2.19	6, 86	0.0519
Time × fertilization × treatment	0.9345	0.49	6, 86	0.8113

(b) Between-tree effects

Source	df	Type III SS	MS	*F*	*P*
Thinning	1	15.8144	15.8144	2.30	0.1360
Fertilization	1	4.2134	4.2134	0.61	0.4375
Treatment	2	109.8397	54.9199	8.00	0.0011
Thinning × fertilization	1	2.1217	2.1217	0.31	0.5810
Thinning × treatment	2	17.5259	8.7630	1.28	0.2889
Fertilization × treatment	2	2.6788	1.3394	0.20	0.8234
Error	45	308.8950	6.8643		

We used artificial fungal inoculations (not natural or induced SPB attacks) in our study and so conclusions on resistance to bark beetles must be interpreted with caution (Lieutier 2002). There are likely important differences between artificial inoculations and natural beetle attacks: for instance, the percentage of SPB carrying *O. minus* spores varies (Hofstetter 2004). However, in some cases, there is close correspondence between results from artificial inoculations and from natural attacks (Guérard et al. 2000, Långström et al. 2001).

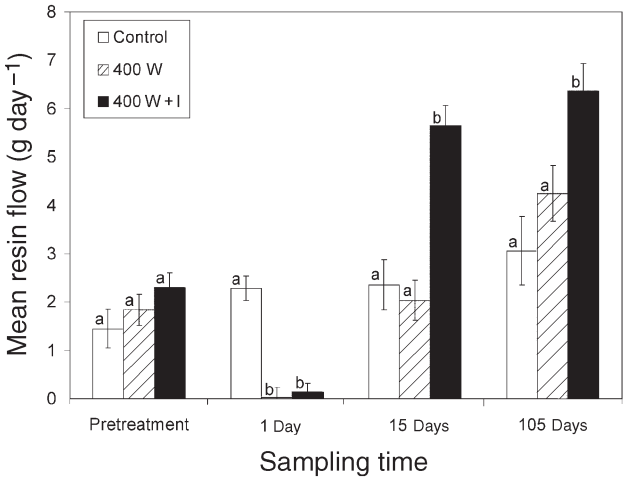

Figure 3. Effects of wounding (W) and inoculation with *Ophiostoma minus* (I) on the yield of oleoresin from a 1.2-cm wound in loblolly pine at Camp Beauregard, Louisiana. Trees were either mass wounded 400 times (400 W), or mass wounded + inoculated (400 W + I). Error bars are standard errors. Identical letters indicate means that are not statistically different within each sampling time.

Based on the demonstrably higher resin flow, we hypothesize, as have others (Lombardero et al. 2000), that beetles attacking previously attacked trees—within the zone of the previous attack—face a more extensive resinous host response than beetles attacking previously unattacked trees. However, based on the limited sampling we conducted one year post-treatment at Camp Beauregard, this effect does not appear to extend beyond a single season.

Ruel et al. (1998), who studied the effects of a different type of mass wounding, demonstrated similar effects on resin yield and showed short-term patterns similar to those we observed. In our study, unwounded trees produced higher resin yields than wounded only or wounded + inoculated trees 1 day post-inoculation, and a recovery of resin defense was noted in both studies at 15 days post-inoculation. These data indicate the mixed effects of mass wounding on loblolly pine. Recently published studies have also indicated that mass wounding can decrease tree resistance to SPB and *O. minus* (Tisdale et al. 2003*a*, 2003*b*). However, these studies did not incorporate simultaneous impacts of wounding and fungal inoculation, as typically occurs in the natural SPB infestations.

We found that neither fertilization nor irrigation significantly affected constitutive resin yield, the initial defense necessary for pitching out SPB. This is contrary to the results of a study conducted 5 years earlier at the same site (Warren et al. 1999), in which fertilization decreased resin yield by up to 50% compared with unfertilized controls. This difference may reflect a difference in tree age (Shrimpton 1973). Fertilization resulted in significant increases in tree growth (Warren et al. 1999) and growth continued to be positively influenced by the

Table 3. Factorial 3-way ANOVA of lesion size data from the SETRES site, North Carolina. A fourth-root transformation was applied to lesion size before analysis to reduce skewness and down-weight large outlying values.

Source	df	Type III SS	MS	*F*	*P*
Irrigation	1	0.82906	0.82906	6.64	0.0105
Fertilization	1	0.74406	0.74406	5.96	0.0152
Treatment	1	17.56071	17.56071	140.56	< 0.0001
Irrigation × fertilization	1	0.40636	0.40636	3.25	0.0723
Irrigation × treatment	1	0.15206	0.15206	1.22	0.2708
Fertilization × treatment	1	0.00898	0.00898	0.07	0.7888
Irrigation × fertilization × treatment	1	0.20600	0.20600	1.65	0.2001

fertilization treatments at the time of our study (Daniel J. Robison, North Carolina State University, personal communication). However, Warren et al. (1999) did not measure induced resin yields, nor did they administer mass inoculation or wounding to trees. In addition, some of their sampling was conducted during a 3-week drought. Although water stress may have affected resin yields in the earlier study, the resin yields obtained in that study (from 0.2 to 1.5 g day^{-1}) were comparable to values (0.6 to 1.5 g day^{-1}) obtained in our study. Dunn and Lorio (1993) found that irrigation increased resin production in loblolly pine following "simulated SPB attack." However, in our irrigated trees, significantly larger fungal lesions developed, regardless of fertilization treatment, indicating greater fungal success (as measured by the area of host tissue colonized before the invasion was stopped by the host's defensive response).

Our results at Camp Beauregard agree with results of previous work at the same site 3–4 years ago (Lombardero et al. 2000). Although the work of Lombardero et al. (2000) was conducted during a severe drought, they found no effects of fertilization treatment on constitutive or wound-induced (by scraping rather than mass inoculation) resin yield. However, induced resin yields reported by Lombardero et al. (2000) (0.8–2.2 g day^{-1}) were substantially lower than those we recorded (1.5– 6.5 g day^{-1}). In contrast to the previous study at SETRES (Warren et al. 1999), Lombardero et al. (2000) found no significant effects of fertilization on tree growth; however, they noted that induced resin flow was highest in faster growing trees, during periods of rapid tree growth. They also hypothesized that mass wounding might increase resin duct filling before there is negative feedback to resin biosynthesis. It seems likely that a similar mechanism was at work in our study, perhaps amplified by the presence of an invading fungus, in the trees with elevated resin flow.

We observed no signs of systemic induced resistance in loblolly pine. Lesions from inoculations outside the mass wounding and inoculation site did not differ between treatments. Fungal success was unaffected by prior fungal inoculation of the host. These results are in agreement with Krokene et al. (1999) who, though reporting induced resistance in Norway spruce, noted this phenomenon only in the immediate vicinity of mass inoculations.

In our study, mass inoculations with *O. minus* caused no tree deaths at either site (a recent study indicates that no trees have died since that time). However, beetles may inoculate with *O. minus* at rates of up to 1900 locations per square meter (Fargo et al. 1978). Although some bark beetle-associated fungi kill trees (Krokene and Solheim 1998, Solheim et al. 1993, Lieutier 2002), numerous inoculation studies have failed to demonstrate a similar role for *O. minus* (e.g., Paine and Stephen 1987, Cook and Hain 1988, Ross et al. 1992). Paine et al. (1997) noted that the long-held assumption that bark beetle-associated fungi kill trees is based primarily on vector relationships, the association of staining with dead trees, and instances of artificial mass inoculation killing trees. However, as Lieutier (2002) has explained, this set of associations does not necessarily indicate that the fungus is responsible for tree death. Rather, the role of bark beetle-associated fungi, e.g., *O. minus*, may be that of "co-factors" (Lieutier 2002, Kopper et al. 2004), defined by Beckage (1998) as a biotic agent that is not itself pathogenic, but facilitates beetle infestation by compromising host defenses.

Consistent with that interpretation, we found that fungal inoculation significantly and dramatically reduced resin flow 1 day after inoculation (Figures 1 and 2). This may be an indication that the tree, in defending against fungal infection, is more vulnerable to beetle infestation. It is during this critical period that beetles first enter a tree and either succeed or fail to trigger aggregation. During beetle aggregation, anything that contributes to the depletion of the host tree's ability to synthesize secondary metabolites improves the probability of successful beetle mass attack (Lieutier 2002). Subsequently, the ultimate death of the tree likely occurs from a combination of bark beetle and fungal effects (Paine et al. 1997, Lieutier 2002).

Our data cast further doubt on the role of *O. minus* as a virulent plant pathogen, as do the complex interactions of *O. minus* with SPB. *Ophiostoma minus* likely aids SPB in overcoming its tree host, and acts as a co-factor by either detoxifying host chemistry or causing necrosis of host tissues. Tree mortality attributed to SPB in the absence of *O. minus* has been noted previously (Hetrick 1949, Bridges et al. 1985), as has blue-staining in living trees (Nebeker et al. 1993). The relative aggressiveness of *O. minus* within tree tissues may aid in

stimulating or overcoming host defenses, or both, benefitting SPBs. However, a virulent fungus may negatively impact its beetle vector. As Lieutier (2002) noted, "if a highly pathogenic blue-stain fungus exhausted a tree's defenses and rapidly killed the tree, it would make the host tissues unsuitable for brood development." This may be especially true in the *O. minus*–SPB association in which the blue-stain fungus is highly antagonistic to SPB mutualistic fungi (Klepzig and Wilkens 1997) and thus to larval development (Barras 1970). The moderately virulent habit of *O. minus* may, ultimately, be the best strategy for SPB: it allows the beetle to mass attack the tree, but limits the extent to which the fungus can grow during the process of beetle development (Lieutier 2002).

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References

Albaugh, T.J., H.L. Allen, P.M. Dougherty, L.W. Kress and J.S. King. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. For. Sci. 44:317–328.

Anderson, T.W. 1958. An introduction to multivariate statistical analysis. John Wiley and Sons, New York, 752 p.

Baker, J.B. and O.G. Langdon. 1990. Silvics of North America: 1. Conifers; 2. Hardwoods. *In* Agriculture Handbook 654, Vol. 2. Eds. R.M. Burns and B.H. Honkala. U.S. Dept. Agric., Forest Service, Washington, DC, 877 p.

Barras, S.J. 1970. Antagonism between *Dendroctonus frontalis* and the fungus *Ceratocystis minor.* Ann. Entomol. Soc. Am. 63: 1187–1190.

Beckage, N.E. 1998. Parasitoids and polydnaviruses—an unusual mode of symbiosis in which a DNA virus causes host immunosuppression and allows the parasitoid to develop. Bioscience 48: 305–311.

Bonello, P. and J.T. Blodgett. 2003. *Pinus nigra-–Sphaeropsis sapinea* as a model pathosystem to investigate local and systemic effects of fungal infection of pines. Physiol. Mol. Plant Pathol. 63: 249–261.

Bonello, P., T.R. Gordon and A.J. Storer. 2001. Systemic induced resistance in Monterey pine. For. Pathol. 31:99–106.

Bridges, J.R., W.A. Nettleton and M.D. Connor. 1985. Southern pine beetle infestations without the blue-stain fungus *Ceratocystis minus*. J. Econ. Entomol. 78:325–327.

Christiansen, E. 1985. *Ips*/*Ceratocystis* infection of Norway spruce: what is a deadly dosage? Z. Ang. Entomol. 99:6–11.

Christiansen, E., P. Krokene, A.A. Berryman, V.R. Franceschi, T. Krekling, F. Lieutier, A. Lonneborg and H. Solheim. 1999. Mechanical injury and fungal infection induce acquired resistance in Norway spruce. Tree Physiol. 19:399–403.

Cook, S.P. and F.P. Hain. 1987. Susceptibility of trees to southern pine beetle, *Dendroctonus frontalis* (Coleoptera:Scolytidae). Environ. Entomol. 16:9–14.

Cook, S.P. and F.P. Hain. 1988. Wound response of loblolly and shortleaf pine attacked or reattacked by *Dendroctonus frontalis* Zimmermann (Coleoptera:Scolytidae) or its fungal associate, *Ceratocystis minor* (Hedgecock) Hunt. Can. J. For. Res. 18:33–37.

Coulson, R.N. 1980. Population dynamics. *In* The Southern Pine Beetle. Eds. R.C. Thatcher, J.L. Searcy, J.E. Coster and G.D. Hertel. USDA Forest Service Tech. Bull. 1631, pp 71–105.

Drooz, A.T. 1985. Insects of eastern forests. Misc. Publication 1426, U.S. Dept. Agric., Forest Service, Washington, DC, 608 p.

Dunn, J.P. and P.L. Lorio, Jr. 1993. Modified water regimes affect photosynthesis, xylem water potential, cambial growth and resistance of juvenile *Pinus taeda* L. to *Dendroctonus frontalis* (Coleoptera: Scolytidae). Environ. Entomol. 22:948–957.

Fargo, W.S., R.N. Coulson, P.E. Pulley, D.N. Pope and C.L. Kelley. 1978. Spatial and temporal patterns of within-tree colonization by *Dendroctonus frontalis* (Coleoptera: Scolytidae). Can. Entomol. 110:1213–1232.

Guérard, N., E. Dreyer and F. Lieutier. 2000. Interactions between Scots pine, *Ips accuminatus* (Gyll.) and *Ophiostoma brunneociliatum* (Math.): estimation of the critical thresholds of attack and inoculation densities and effects on hydraulic properties in the stem. Ann. For. Sci. 57:681–690.

Hepting, G.H. 1947. Stimulation of oleoresin flow in pines by a fungus. Science 105:209.

Hetrick, L.A. 1949. Some overlooked relationships of southern pine beetle. J. Econ. Entomol. 42:466–469.

Hodges, J.D., W.W. Elam, W.F. Watson and T.E. Nebeker. 1979. Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks. Can. Entomol. 111:889–896.

Hofstetter, R.W. 2004. Interspecific interactions and population dynamics of the sounthern pine beetle. Dartmouth College, Hanover, NJ, 184 p.

Horntvedt, R., E. Christiansen, H. Solheim and S. Wang. 1983. Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. Medd. Nor. Inst. Skogforsk. 38:1–20.

Huynh, H. and L.S. Feldt. 1970. Conditions under which mean square ratios in repeated measurements designs have exact *F*-distributions. J. Am. Stat. Assoc. 65:1582–1589.

Jacobs, K. and T. Kirisits. 2003. *Ophiostoma kryptum* sp. nov. from *Larix decidua* and *Picea abies* in Europe, similar to *O. minus*. Mycol. Res. 107:1231–1242.

Klepzig, K.D. and R.T. Wilkens. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. Appl. Environ. Microbiol. 63:621–627.

Klepzig, K.D., J.C. Moser, M.J. Lombardero, R.W. Hofstetter and M.P. Ayres. 2001. Symbiosis and competition: complex interactions among beetles, fungi and mites. Symbiosis 30:83–96.

Kopper, B.J., K.D. Klepzig and K.F. Raffa. 2004. Components of antagonism and mutualism in *Ips pini*—fungal interactions: relationship to a life history of colonizing highly stressed and dead trees. Environ. Entomol. 33:28–34.

Kramer, C.Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12:307–310.

Krokene, P. and H. Solheim. 1998. Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. Phytopathology 88:39–44.

Krokene, P. and H. Solheim. 2001. Loss of pathogenicity in the blue-stain fungus *Ceratocystis polonica*. Plant Pathol. 50: 497–502.

Krokene, P., E. Christiansen, H. Solheim, V.R. Franceschi and A.A. Berryman. 1999. Induced resistance to pathogenic fungi in Norway spruce. Plant Physiol. 121:565–569.

Krokene, P., H. Solheim and B. Långström. 2000. Fungal infection and mechanical wounding induce disease resistance in Scots pine. Eur. J. Plant Pathol. 106: 537–541.

Långström, B., H. Solheim, C. Hellqvist and P. Krokene. 2001. Host resistance in defoliated pine: effects of single and mass inoculations using bark beetle-associated blue-stain fungi. Agric. For. Entomol. 3:211–216.

Lieutier, F. 2002. Mechanisms of resistance in conifers and bark beetle attack strategies. *In* Mechanisms and Deployment of Resistance in Trees to Insects. Eds. M.R. Wagner et al. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 31–77.

Lombardero, M.J., M.P. Ayres, P.L. Lorio, Jr. and J.J. Ruel. 2000. Environmental effects on constitutive and inducible resin defenses of *Pinus taeda*. Ecol. Lett. 3:329–339.

Lombardero, M.J., M.P. Ayres, R.W. Hofstetter, J.C. Moser and K.D. Klepzig. 2003. Strong indirect interactions of *Tarsonemus* mites (Acarina, Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). Oikos 102:243–252.

Lorio, P.L., Jr. 1986. Growth-differentiation balance: a basis for understanding southern pine beetle–tree interactions. For. Ecol. Manage. 14:259–273.

Lorio, P.L., Jr. and R.A. Sommers. 1986. Evidence of competition for photosynthates between growth processes and oleoresin synthesis in *Pinus taeda* L. Tree Physiol. 2:301–306.

Morrison, D.F. 1976. Multivariate statistical methods. 2nd Edn. McGraw-Hill, New York, 480 p.

Nebeker, T.E., J.D. Hodges and C.A. Blanche. 1993. Host reactions to colonization by bark beetles and associated pathogens. *In* Interactions Among Bark Beetles, Pathogens and Conifers in North American Forests. Eds. T. Schowalter and G. Filip. Academic Press, London, pp 157–173.

Paine, T.D. and F.M. Stephen. 1987. Response of loblolly pine to different inoculum doses of *Ceratocystis minor*, a blue-stain fungus associated with *Dendroctonus frontalis*. Can. J. Bot. 65: 2093–2095.

Paine, T.D., K.F. Raffa and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi and live host conifers. Annu. Rev. Entomol. 42:179–206.

Popp, M.P., J.D. Johnson and T.L. Massey. 1991. Stimulation of resin flow in slash and loblolly pine by bark beetle vectored fungi. Can. J. For. Res. 21:1124–1126.

Raffa, K.F. and A.A. Berryman. 1983. Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Can. Entomol. 115:723–734.

Ross, D.W., P. Fenn and F.M. Stephen. 1992. Growth of southern pine beetle associated fungi in relation to the induced wound response in loblolly pine. Can. J. For. Res. 22:1851–1859.

Ruel, J.J., M.P. Ayres and P.L. Lorio, Jr. 1998. Loblolly pine responds to mechanical wounding with increased resin flow. Can. J. For. Res. 28:596–602.

SAS Institute. 1999. SAS/STAT User's guide, Version 8. SAS Institute, Cary, NC, 3848 p.

Schultz, R.P. 1997. Loblolly pine: the ecology and culture of loblolly pine (*Pinus taeda* L.). USDA Forest Service, Southern Forest Expt. Stn., Agric. Handbook No. 713. New Orleans, LA, 493 p.

Shrimpton, D.M. 1973. Age- and size-related response of lodgepole pine to inoculation with *Europhium clavigerum*. Can. J. Bot. 51: 1155–1160.

Solheim, H., B. Langstrom and C. Hellqvist. 1993. Pathogenicity of the blue-stain fungi *Leptographium wingfieldii* and *Ophiostoma minus* to Scots pine: effect of tree pruning and inoculum density. Can. J. For. Res. 23:1438–1443.

Strom, B.L., R.A. Goyer, L.L. Ingram, Jr., G.D.L. Boyd and L.H. Lott. 2002. Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle. For. Ecol. Manage. 158:169–178.

Tisdale, R.A., T.E. Nebeker, and J.D. Hodges. 2003*a*. Role of oleoresin flow in initial colonization of loblolly pine by southern pine beetle. J. Entomol. Sci. 38: 576–582.

Tisdale, R.A., T.E. Nebeker and J.D. Hodges. 2003*b*. The role of oleoresin flow in the induced response of loblolly pine to a southern pine beetle associated fungus. Can. J. Bot. 81:368–374.

Trapp, S. and R. Croteau. 2001. Defensive resin biosynthesis in conifers. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52:689–724.

Warren, J.M., H.L. Allen and F.L. Booker. 1999. Mineral nutrition, resin flow and phloem phytochemistry in loblolly pine. Tree Physiol. 19:655–663.

Wear, D.N. and J.G. Greis. 2002. Southern forest resource assessment. Gen. Tech. Rep. SRS-53. U.S. Dept. Agric., Forest Service, Southern Res. Stn., Asheville, NC, 635 p.